THE GROWTH CYCLE OF CAMBIUM AND THE STRUCTURE OF THE VASCULAR TISSUE IN THE CORM OF *ISOETES TAIWANENSIS*

SU-HWA TSAI CHIANG(1)

Abstract: The structure of the vascular tissues and the origin as well as the seasonal growth of the vascular cambium in the corm of *Isoetes taiwanensis* have been studied. A periodical change in the cambial activity was observed, and the presence of an extensive one-banded secondary thickening tracheary elements in secondary vascular tissues was noted.

INTRODUCTION

Isoetes taiwanensis, was recently discovered in Taiwan (DeVol, 1972). This peculiar plant, *Isoetes*, has provoked much interest among many botanists for a long time. As the matter of fact, there still is some difference of opinion on the anatomy of the corm. As pointed by earlier workers these may be due to structural variations in different species or specimens. Many investigators have attained more general agreement on the structures of the primary xylem and the secondary cortex among the various tissues in the corm of *Isoetes* (Farmer, 1890; Paolillo, 1963; Scott and Hill, 1900; Smith, 1900; Stokey, 1909; West and Takeda, 1915). A preliminary study on the vascular elements as well as the arrangement of the various tissues in the corm of *I. taiwanensis* was published in the previous issue of this journal (Yang, Chiang & DeVol, 1975). It seems to the present worker that it is worthwhile to make a more detailed observation on it because of the reasons mentioned above. The structures of the primary phloem, secondary vascular tissue (prismatic layer) and the activity of the cambium are emphasized in the present investigation since these structures have caused much difference of opnion.

MATERIALS AND METHODS

The plants used were collected from Seven Star Mountain (Chi-hsin-shan, 七星山), and transferred to the water tanks in shade house of the Botany Department, National Taiwan University for more than six months before the biginning of this investigation. The plants were potted and submersed. The tanks were kept full of water by slow flowing tap water.

The materials for histological examination, corms of about 1 cm in diameter were collected twice each month, respectively on the 10th and 25th from June, 1972 to December, 1973. Two corms were obtained each time. All the corms used for sectioning were three-lobed, and sections were made longitudinally both perpendicular and parallel to the furrow of the same section (Fig. 1), at 8 to 10 u. Transverse sections were also prepared for other purposes. Microslides were prepared by the usual paraffin method, and paraffin ribbons from each corm were divided into two groups at the middle. One group was stained with safranin and fast-green (Johansen, 1940), and the other with orange G, tannic acid, and iron alum (Sharman, 1943).

The daily water temperature of the culture tanks was recorded at noon during the course of the experiment.

(1) 江蘇淑華, Professor of Botany, National Taiwan University.

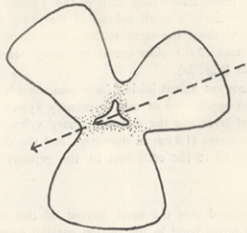


Fig. 1. Semidiagramatic drawing of a corm from the transverse section showing the plane (arrow) of section for histological examination.

RESULTS

Apical meristem

As seen in the median longitudinal plane of a corm, the apical meristem is found at the apex of the corm (Fig. 2). No single apical cell such as is commonly found in other pteridophytes can be identified. The apical meristem consists of a group of cells which are richer in protoplasm and more thin-walled than the cells in the mature tissues. The apical meristems of the older corms i. e. those of more than 1 cm in diameter always possess a single superficial layer of protoplasmic-rich cells, rarely of two layers. But there is no evidence of a tunica, since they divide in various planes such as: anticlinally, periclinally and the plane between

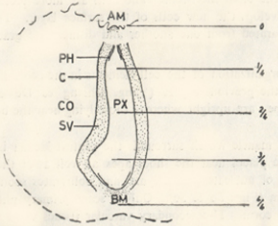


Fig. 2. Diagram of the median longitudinal section of a corm, the corm is divided into four parts. The cambial cells are counted at three places indicated as 1/4, 2/4, 3/4 of a corm.
Key to labelling: AM: shoot apical meristem; BM: basal meristem; BT: one-banded tracheid; C: cambium; CO: cortex; LT: leaf trace; PH: primary phloem:; PX: primary xylem; SV: secondary vascular tissue.

both (Fig. 3b, arrow). The protoplasmic-rich cells in the young corms i. e. those less than 0.3 cm in diameter, occupy relatively a small volume of the shoot spices (Fig. 3a). But the size of the apical inital cells are of about the same as that observed in the older corms (Figs. 3a & 3b). Mitotic figures in this area of the older corms are rarely seen whereas they are usually abundant in the young corms (Fig. 3a).

At a certain distance from the apical initials (protoplasmic-rich cells) down along the corm axis, there is the very sudden appearance of mature primary xylem elements. The cell layers between the superficial apical layer and the mature primary xylem are always less than 15 cells away even in the very large corms (1.8 cm in diameter), and the degree of vacuolation in this area from the second layer down to the cells next to the primary xylem is not conspicuous (Figs. 3a & 3b).

Basal meristem

The basal meristem is located over the basal furrow of the central primary xylem core (Fig. 2). So it appears as a broad band in the section parallel with the furrow of the corm (Fig. 3d), and shows a narrower W-shape when cut perpendicular to the corm furrow (Fig. 3c). As shown in Fig. 3d, the basal meristematic cells are short and flat and compactly arranged. Their transverse walls are regularly arranged in rows and the longitudinal walls in the same row occur in orderly lines. Apparently they mainly divide transversely. When they divide longitudinally they form new walls parallel with the furrow plane less frequently than the other planes, since the orderly arranged pattern of the basal meristematic cells are not distinct as seen in the plane perpendicular to the furrow (Fig. 3c).

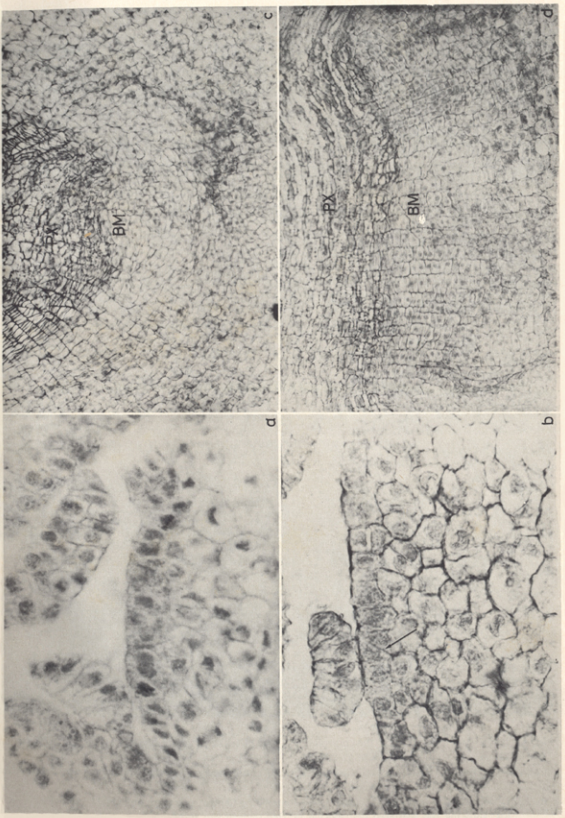
The cell layers of the basal meristem vary in relation to the age (or size) of the plant. The large corms have a thicker basal meristem whereas the smaller corms have thinner ones. In 2 cm corms for example, the meristem is about 20 cell-layers, whereas in a 0.3 cm corm it only has two cell-layers. This is like the shoot apex, in that the well developed tracheids always lie very near to the meristematic cells (Figs. 3c & 3d). This condition is quite common in all slides examined. The daughter cells formed by the basal meristem mature into the primary xylem upwards and into the surface layer of the basal furrow downwards (Fig. 2). The surface layer is situated outside the basal meristem. The cells are more loosely arranged and cover the basal meristem which develops the new cells of the surface layer. The outermost cells of the surface layer become separated from one another and disintegrate gradually.

Primary vascular tissue

The kinds and the organization of the cell elements in the centrally located primary vascular tissue were described in the previous report (Yang, Chiang & DeVol, 1975). The tracheids located near the shoot apex are upright whereas those lying near the basal meristem are typically procumbent (Fig. 3d).

The so called phloic mantle which surrounds the central lacuna is not conspicuous. It can only be recognized at the places near the shoot apex as well as at the basal meristem (Figs. 2, 5b, 5c). It consists only of nucleate parenchyma, and obliterates soon after the lacunation of the central primary xylem (Figs. 5b & 5c). Only a scant primary phloem layer can be seen in the middle region of the corm. The secondary vascular tissues come to lie immediately next to the central primary xylem lacuna (Fig. 2).

Fig. 3. Median longitudinal sections through the apical meristems (a & b, ×820), and basal meristems (c & d, ×160).
(a) from a young corm of 0.3 cm in diameter.
(b) from an old corm of 1.5 cm in diameter.
(c) through the plane perpendicular to the furrow in an old corm.
(d) through the plane parallel with the furrow.


c
BM
d
PX
BM
d
b

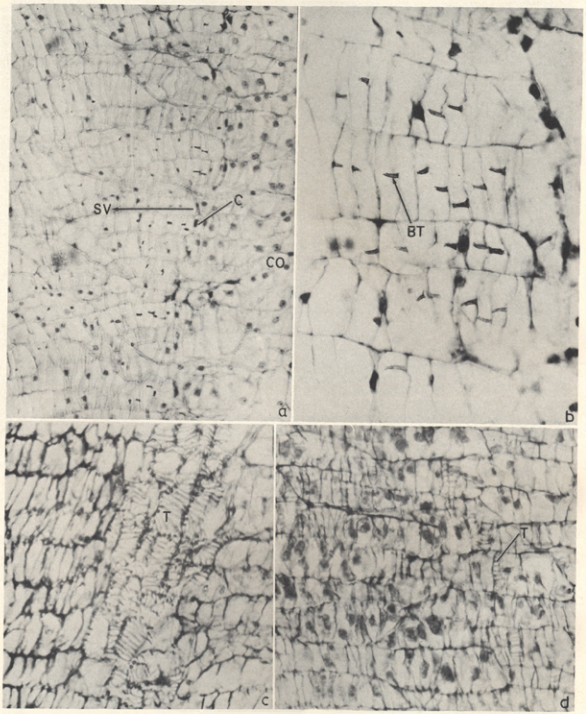


Fig. 4. Radial sections showing the morphology and distribution of tracheids in secondary vascular tissue. (a)×150 (c) (d)×320; (b)800.
(a & b) with safranin and fast-green stain emphasizing the one banded secondary thickened tracheids by using the green filter.
(c) showing a part of the linearly distributed ring-helicle secondary thickened tracheids.
(d) showing the scattered distribution of ring-scalariform tracheids.

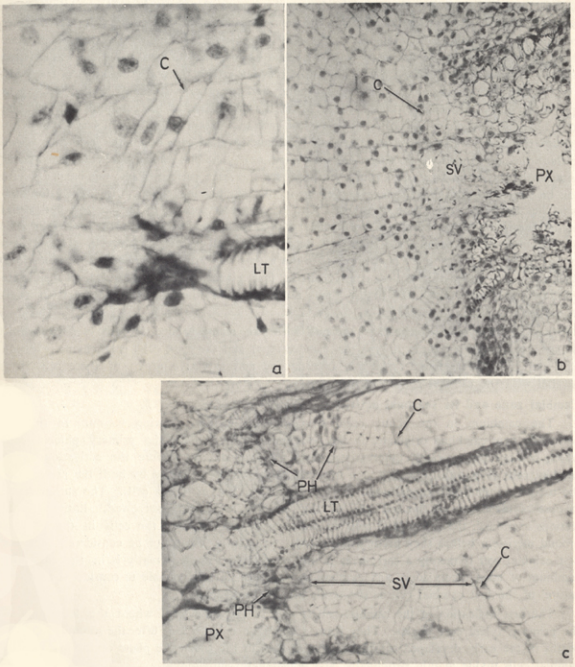


Fig. 5. Longitudinal sections of the corms: near the apical meristem, showing the origin (arrow) of cambial cells (a, ×800); from a young corm of 0.2 cm in diameter (b, ×315), and near the leaf traces in an old corm (c, ×315), note the close arrangement of the perforated primary xylem cores to the secondary xylem (the cells in this region have one banded secondary walls).

Secondary vascular tissue

The secondary vascular tissue (or prismatic layer) consists of three kinds of cell elements. These are the parenchyma (mostly containing starch), sieve cells and tracheids. Their morphological characteristics were mentioned in the previous paper (Yang *et al.*, 1975). The secondary vascular tissue is situated laterally outside the central primary vascular tissue (Fig. 2). Further examination of the various developing stages of the corms reveals that the distribution of these three kinds of cells in the secondary vascular tissue is somewhat different from that was reported earlier (Yang *et al.*, 1975). As mentioned in the previous article, there are two kinds of tracheids, i. e., the one with one banded secondary thickening and the other with many banded (or ring to spiral) secondary thickening. In the young plant, the derivatives of the cambium only give rise to one banded tracheids (Fig. 5b). Their cell walls are thin except at the region bearing the one-banded secondary walls. They are likely to be thinnest-walled cells of all the elements if we exclude the one-banded region (Figs. 4a, 4b, 5b, 5c, 6c). The one-banded tracheids always occur as extensive layers in most specimens. Furthermore the same pattern of the one-banded nature can be seen in the transverse plane as well as in the longitudinal section. Apparently the one-band lies in the central region of the cell with its long axis parallel to the horizon and radial to the corm. The 'one-band' of the secondary thickening can hardly be seen in all the cells of the same section, because the band only occurs in a small protion of the cell. They always appear as broken, incomplete pieces. So that the extensively grouped one-banded tracheids look the widely distributed thin-walled, empty cells at first glance. The one-banded tracheids are most frequenly seen type in all of the plants examined. No other type of tracheid is so commonly seen in the sections (Figs. 4a, 4b, 5b, 5c).

The second type of tracheid, ie, the one with the many banded secondary wall, mainly occurs scattered among the first type (one-banded type), and the sieve cells parenchyma (Fig. 4d). Only two plants were found to have a continuouns and extensive group of these cells lying parallel with the cambial zone (Fig. 4c).

Cambial zone and its seasonal activity

The cambium jn the young corm develops early in first growing season. In the old corm, the new cambium originates in the cells immediately outside the primary phloem cells which can not be accurately differentiated from the ground tissue before the activation of the cambial cells (Fig. 5a). The first sign of the cambial cell is identified by both the presence of the narrow cambial cell or cells and the topographic nature of these cells. The author failed to observe the course of the first step of the cambial cells being narrowed. But from the sections examined, the procambial cells do not remain narrower than the cells in the primary ground meristem before they become lacunated. Apparently it can not be considered that the first cambial cell is merely a carry-over of the procambium as it normally is in the fascicular cambium of most dicotyledons and gymnosperms. It is more reasonable to consider the 'narrow nature' of the first cambial cell as the result of a cell division.

The first indication of the cambial cell is seen at the same level where the lacunation of the primary xylem core begins. They form a complete cylinder long after the secondary growth begins in the early developed cambial region as seen in the transverse plane of the corm. The cambial cells divide mainly periclinally, and occasionally anticlinally.

The cambial cells show the same appearance in both transverse and longitudinal planes. The cambial initials are radially very short, but tangentially and vertically isodiametric. They are located between the secondary vascular tissue and secondary cortex. In other words, the cambium gives rise to the secondary vascular tissue inward and to secondary cortex outward. The cambial zone is well defined when it is in the actively dividing stage. During the active period of the cambium a group of flattened cambial initials are arranged in radial dimensions

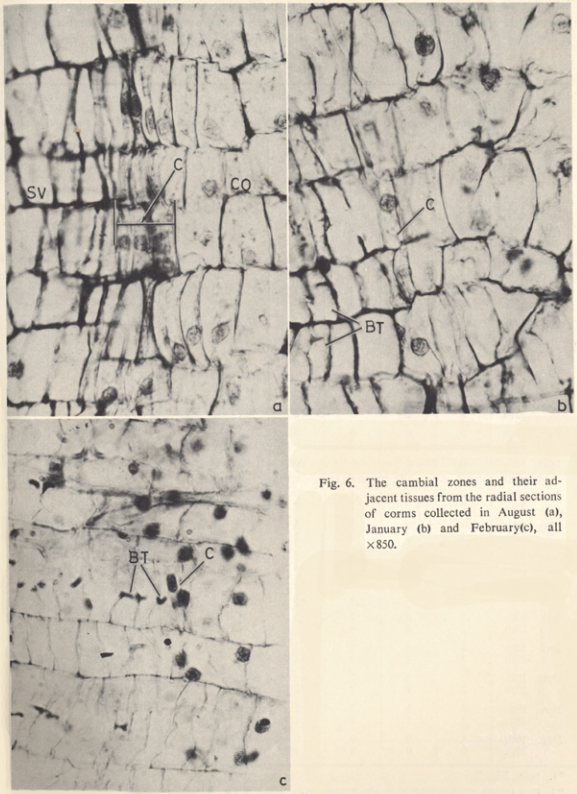


Fig. 6. The cambial zones and their adjacent tissues from the radial sections of corms collected in August (a), January (b) and February(c), all ×850.

and possess denser protoplasms (Fig. 6a). And there are several layers of non-mature derivatives of the cambium. The gradual transition from the meristematic cambial initials to the mature cells can be traced. But in the corms which are collected in the winter or in the less active season, this histological pattern can not be observed. The protoplasms in the cambial cells are lighter and the continuously arranged flattened pattern of cambial cells is not visible. They

Table 1. Variation in number of initial cells in cambial zone

Date		*1 Number of initial cells						average	sporangia	
		1/4		2/4		3/4			young	mature
		*2corm 1	corm 2	corm 1	corm 2	corm 1	corm 2			
1972										
June	10th	3	3	3	3	3	2	2.83	+	+
	25th	3	3	3	4	3	4	3.33	+	+
July	10th	4	4	4	5	4	5	4.33	+	+
	25th	4	4	4	5	4	4	4.16	+	+
Aug.	10th	6	3	4	3	3	4	3.63	+	+
	25th	2	5	4	4	3	5	3.63	+	+
Sep.	10th	3	2	4	3	4	3	3.16	−	+
	25th	3	2	3	3	3	3	2.83	−	+
Oct.	10th	3	3	3	3	2	3	2.83	−	+
	25th	3	—	3	—	3	—	3.00	−	+
Nov.	10th	1	2	3	2	2	3	2.16	(+)	−
	25th	1	0	1	2	1	2	1.16	−	+
Dec.	10th	1	1	0	1	0	1	0.66	−	+
	25th	0	1	0	0	0	1	0.33	−	−
1973										
Jan.	10th	0	0	0	0	0	1	0.16	−	+
	25th	0	0	0	0	1	0	0.16	−	−
Feb.	10th	0	0	0	0	1	0	0.16	−	−
	25th	0	0	0	0	0	1	0.16	−	−
Mar.	10th	0	0	1	0	0	0	0.16	+	+
	25th	1	0	1	0	0	0	0.33	+	+
Apr.	10th	0	1	1	0	1	0	0.50	+	+
	25th	2	2	2	2	3	1	2.00	+	+
May	10th	3	3	3	3	3	3	3.00	+	+
	25th	3	3	3	2	3	3	2.83	+	+
June	10th	3	4	3	3	3	4	3.33	+	+
	25th	4	3	4	4	4	4	2.63	+	+
July	10th	4	5	5	4	4	4	4.33	+	+
	25th	4	4	4	4	4	4	4.00	+	+
Aug.	10th	5	4	4	5	4	3	4.16	−	+
	25th	5	4	4	4	4	4	4.16	−	+
Sep.	10th	3	3	4	2	3	3	3.00	−	+
	25th	3	3	3	3	3	2	2.83	−	+
Oct.	10th	2	3	2	3	2	2	2.33	−	−
	25th	2	1	2	2	1	2	1.66	−	+
Nov.	10th	1	2	3	2	1	2	1.83	(+)	+
	25th	1	1	1	1	1	1	1.00	−	+
Dec.	10th	1	0	0	0	0	1	0.50	−	−
	25th	0	0	0	1	0	0	0.16	−	−

*1. refer Fig. 2.

*2. two corms were collected in the same day.

Table. 2. Temperature variation of water in culture tanks at noon during the experiment (°C)

Date	Monthly average temperature	Data	Monthly average temperature
1972 June	25.2	1973 Apr.	20.3
July	26.3	May	23.0
Aug.	27.0	June	25.2
Sep.	26.0	July	27.2
Oct.	23.1	Aug.	27.1
Nov.	20.0	Sep.	25.8
Dec.	15.0	Oct.	22.0
1973 Jan.	12.9	Nov.	18.0
Feb.	15.8	Dec.	16.8
Mar.	17.1		

appear to be flattened in some places, whereas they are more or less isodiametric in others such as that of the secondary cortical cells (Fig. 6b). So the dormant cambium consists of a narrow zone (or none at all) of radially flattened cells. But in some other places, the cambial initials lie immediately next to the mature tracheids (always those with the one-banded secondary thickening) (Fig. 6c).

No sign of the radial swelling of the cambial cells preceeded the division. The radial expansion of the cells in cambial zone always falls behind the repeated periclinal divisions in the active season. Consequently the cambial zone appeared as a series of very narrow cells compactly arranged in radial files (Fig. 6a).

By counting the numbers of cell layers of the cambial initials, it is revealed that the structure and activity of the cambium in the corm shows great variation according to the different seasons (Table 1). The data of the numbers of initial layers shown in Table 1 were obtained

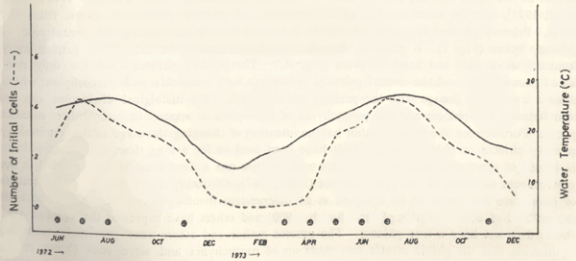


Fig. 7. Graph showing the seasonal variations of the cambial activity (black line) and the temperature of water (dotted line) in which the plants grew, ⊕ represents the presence of young sporangium during this period.

from the three regions designated as 1/4, 2/4, 3/4 in the median longitudinal sections collected in the different seasons indicated (Fig. 2). The cambial initials became activated in the beginning of April, countinued for several months, showed its maximum growth in July, and ceased in November (Table 1; Fig. 7). The maximum average number of cell layers of the cambial intials was shown in July to August. It was counted as 4.33. During this investigation, July to August had the highest temperature of the water in which the plants grew (Table 2; Fig. 7). This annual periodicity shows the typical sigmoid growth and was clearly correlated with the change in the water temperature.

The seasonal change of sporangial formation

The presence of the adaxial sporangium was also examined. Five plants were studied on each collecting day for observing the presence of the sporangium. Most plants collected during this investigation bore mature sporangia. It is easy to imagine that the bearing of the mature sporangia can not exactly indicate the formation of sporangium in the stage of collection. The course of maturation to disintegration of a sporangium may take a considerably long time. For this reason, in the present data no account was taken of the mature sporangium. Only the young sporangia were taken into consideration for the confirmation of the time of sporangial formation.

Sporangium began to be formed in March, (which is slightly before the reactivation of the cambial intials, Fig. 7), and continued for several months as the cambium gradually increased its activity, and ceased as soon as the cambium reached its maximum stage of growth. No sign of sporangial formation was seen during the period of the gradual decline of the cambial activity except for the occasional finding of a few specimens collected in November.

DISCUSSION

Both apical and basal meristems contribute to the production of the primary tissue in the corm. The interpretation of the primary xylem core has received more uniform treatment by the ealier as well as the recent workers. It consists of mingled tracheids and parenchyma. The structure of the primary phloem is obviously not complicated but it has not received the same general agreement in interpretation. Most workers have stated that the primary phloem occurs in the corm (Eames, 1936; Foster & Gifford, 1974; Lang, 1915; Paolillo, 1963; Yang *et al.*, 1975), whereas Scott and Hill (1900) reported that primary phloem is absent from the corm. Primary phloem in *Isoetes taiwanensis* is located lateally surrounding the central core of primary xylem (Fig. 2). It gradually becomes smaller in amount in the region further away from the shoot apex and basal meristem (Fig. 5C). The primary phloem consists of only nucleated parenchyma, and the central primary xylem has both tracheids and parenchyma which show a continuous lineage with the primary phloem cells. No histolgical distinction can be seen between the phloem and the parenchyma of the xylem as appears in many other vascular plants. Furthermore the present author has no intention of changing the usage of the terminology primary phloem or phloic mantle which have been used so for a long time, though the phloem is devoid of sieve area bearing cells. However, neither can a sieve area be distinguished in the protophloem elements of the gymnosperms (Fahn, 1967). Obviously the primary phloem elements of *Isoetes* are too simple to be accepted as an independent tissue—phloem, when compared with the phloem of other vascular plants. Smith (1900) and others have expressed the opinion that *Isoetes* does not have a true phloem. The present author and others were previously mistaken in thinking that the phloic mantle was made up of parenchyma and sieve cells (Yang, *et al.*, 1975). Most of the cells described as phloic mantle in the previous paper (Yang *et al.*, 1975) are a part of the secondary vascular tissue. Because the early formed secondary vascular tissue contains so many one-banded tracheids which are not well defined and are seen to be thin-walled

empty cells. The one-banded pattern is always missing during sectioning or too tiny and fragmentary to be identified.

No tissue in *Isoetes* has been the subject of so much discussion as the secondary vascular tissue. It has been reviewed thoroughly by Paolillo (1963). Studying four species of *Isoetes*, Stokey (1909) stated that there were five kinds of cells in the secondary vascular tissue. Scott and Hill (1900) and Paolillo (1963) found three kinds of cells but no empty cells, and Mohl (1845) considered the whole as undifferetiated parenchyma. And some others stated that tracheary elements were absent (West and Takeda, 1915). The vascular elements and the pattern of development of the secondary growth probably varies according to the species. Paolillo (1963) has reported that the secondary vascular tissue formed by the cambium in the young plants consisted of sieve cells only. But this is not the case with the present species. The cambium forms one-banded tracheary elements only in the young corm of *I. taiwanensis* (Fig. 5b). The one-banded tracheid in *I. taiwanensis* may be compared to the empty cells described by Stokey (1909). The appearance of this simplest type of tracheid (one-banded type) in the secondary tissue may imply some unknown but importane evolutional significance in *Isoetes*—one of a few living pteridophytes which represent the secondary growth.

As mentioned above, the cambium is initiated in the parenchyma outside the primary phloem which consists of only nucleate parenchyma. Though the presence of the cambial cells and the secondary tissue can not be confirmed by the occurrence of the radial seriateion alone (Esau, 1943; Paolillo, 1963), the existence of the one-banded tracheids as well as the radially seriated arrangement of them and of their adjacent cortical cells demonstrates the very early origin and the activity of the cambial cells in this species. It interests the present author that the one-banded tracheids occur much more frequently than the other type of the thracheids in the specimens examined.

It is known that both internal and external factors produce a complex of interrelated influences which affect cambial growth. Thus the effects of any one factor is not easy to assess. An aquatic plant would be an excellent material for studying the unifactor affecting cambial activity. In contrast with other types of habitat, water provides a more uniform habitat. The factor that influences aquatic plants most is water temperature, and it is easier to keep water under the fairly uniform condition. Though the periodic annual increments can not be traced in the well developed corm, the activity of cambial cells is periodic rather than continuous. The present observation reveals that the growth of the cambium in *Isoetes taiwanensis* is correlated with seasonal change, mainly due to temperature of the water in which the plants grow. The maximum cambial activity was shown in July to August when the water temperature represents the highest in the year (Fig. 7). The water temperature is always lower than the air temperature in the same location (Lu and Chiang, 1975). It affects the cambial activity directly. The fruiting period shown in Fig. 7 only indicated the presence of very young sporangial bearing sporophylls which were located near the shoot apex. The mature sporangia were omitted because this investigation was not detailed enough to determine how long it would take for a sporangium to mature. Both young and mature sporangia were seen in early March but none were found in late February (Table 1). This may suggest that the developing period of a sporangium from a very young stage to the ripened stage takes less than half a month (time interval of each collection in this investigation). It is very possible that the fruiting period was about one month or more earlier than those growing in the mountain lake from which we obtained the plants (DeVol, 1972).

LITERATURE CITED

DeVol, C. E., 1972. *Isoetes* found on Taiwan. Taiwania 17: 1-7.

EAMES, A. J., 1936. Morphology of vascular plants. Lower groups. McGraw-Hill Co. Inc.

ESAU, K., 1943. Ontogeny of the vasclar bundle in *Zea mays*. Hilgardia **15**: 327–388.

FAHN, A., 1967. Plant Anatomy. Pergamon Press.

FARMER, J. B., 1890. On *Isoetes lacustris*. Ann. Bot. **5**: 37–62.

FOSTER, A. S. & E. M. GIFFORD, 1974. Comparative morphology of vascular plants. 2nd ed. San Francisco Freeman & Co.

LANG, W. H., 1915. Studies on the morphology of *Isoetes*. II. The analysis of the stele of the shoot of *Isotes lacustris* in apical development. Mem. Proc. Manchar. Lit. Phil. Soc. **59**: 29–57.

LU, C. I. & S. H. T. CHIANG, 1975. Seasonal activity of the cambium in the young branch of *Liquidambar formosana*. Taiwania **20**: 32–47.

MOHL, H. von, 1845. Ueber den Bau des Stammes von *Isoetes lacustris*. Linnaea **14**: (cited by West & Takeda, 1915).

PAOLILLO, D. J. JR., 1963. The developmental anatomy of *Isoetes*. III. Biol. Monogr. 31. The Univ. Illinois Press. Urbana.

SCOTT, A. H. & G. T. HILL, 1900. The structure of *Isoetes hystrix*. Ann. Bot. **14**: 413–454.

SMITH, R. W., 1900. The structure and development of the sporophyll and sporangia of *Isoetes*. Bot Gaz. **29**: 225–258.

STOKEY, A. G., 1909. Anatomy of *Isoetes*. Bot. Gaz. **47**: 311–335.

WEST, C. & H. TAKEDA, 1915. On *Isoetes joponica* A. Br. Linn. Soc. London Trans. Bot. II, **8**: 333 –376.

YANG, T. Y., S. H. T. CHIANG & C. E. DEVOL, 1975. Vascular elements in the corm of *Isoetes taiwanensis*. Taiwania **20**: 99–106.